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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER

SGAGIAS, MAGDALENE K

ART UNIT

PAPER NUMBER

1632

NOTIFICATION DATE

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ELECTRONIC

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Chgpatent@leydig.com

<b>Office Action Summary</b>	<b>Application No.</b> 10/594,864	<b>Applicant(s)</b> SHINOHARA ET AL.	
	<b>Examiner</b> Magdalene K. Sgagias	<b>Art Unit</b> 1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

### Status

- 1) ☒ Responsive to communication(s) filed on 18 March 2010.
- 2a) ☐ This action is **FINAL**.                      2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

### Disposition of Claims

- 4) ☒ Claim(s) 1-12, 15, 16, 35 and 36 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1-12, 15, 16, 35 and 36 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 29 September 2006 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

### Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All    b) ☐ Some \*    c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

### Attachment(s)

- |   |   |
|---|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)   | 4) <input type="checkbox"/> Interview Summary (PTO-413)<br>Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)  | 5) <input type="checkbox"/> Notice of Informal Patent Application                       |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)<br>Paper No(s)/Mail Date <u>03/18/2010</u> . | 6) <input type="checkbox"/> Other: _____  |

### **DETAILED ACTION**

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 03/18/2010 has been entered.

Applicant's arguments filed 03/18/2010 have been fully considered. Claims 1-12, 15-16, 35-36 are pending and under consideration. The amendment and newly added claims has been entered. Claims 13-14, 17-34 have been canceled.

#### ***Claim Rejections - 35 USC § 112/Necessitated by Amendment***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The rejection of claims 1-12, 15-16 under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for producing multipotent germline stem (mGS) cells, which comprises culturing testis cells using medium containing glial cell derived neurotrophic factor (GDNF), wherein the testis cells contain spermatogonial stem cells (SSCs), and wherein the testis cells are derived from a postnatal mouse, and isolating multipotent germline stem cells expressing SSEA-1, Forsman antigen,  $\alpha$ 1-integrin,  $\alpha$ 6-integrin, EpCAM, CD9, EE2 and c-kit markers, does not reasonably provide enablement for producing pluripotent stem cells derived from postnatal testis of all mammalian species including human species, forming the three somatic lineages is withdrawn in view of the amendment.

Claims **1-12, 15-16, 35-36** are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for producing multipotent germline stem (mGS) cells, which comprises culturing testis cells using medium containing glial cell derived neurotrophic factor (GDNF) for at least 3 to 6 weeks, wherein the testis cells contain spermatogonial stem cells (SSCs), and wherein the testis cells are derived from a postnatal mouse, and isolating multipotent germline stem cells expressing SSEA-1, Forsman antigen, 1-integrin, 6-integrin, EpCAM, CD9, EE2 and c-kit markers, does not reasonably provide enablement for producing pluripotent stem cells derived from postnatal testis of all mammalian species including human species, forming the three somatic lineages.

The claims are directed to a method of producing pluripotent stem cells, which comprises culturing testis cells using a medium containing glial cell derived neurotrophic factor (GDNF) or an equivalent thereto for at least 3 to 6 weeks, wherein the testis cells contain spermatogonial stem cells, and wherein the testis cells are derived from a postnatal mammal, and isolating pluripotent stem cells from the cultured testis cells.

The claims are broad in scope, encompassing a method that would embrace maintaining pluripotency of any mammalian species or human pluripotent cells by culturing testis cells using a medium containing GDNF for at least 3 to 6 weeks and embodiments, wherein the medium contains, LIF, bFGF, feeder cells and the testis cells are p53-deficient. The disclosure provided by the applicant, in view of the prior art, must encompass a wide area of knowledge to a reasonably comprehensive extent. In other word each of those, aspect considered broad must be shown to reasonable extent so that one of the ordinary skills in the art at the time of the invention by applicant would be able to practice the invention without any undue burden being on such artisan. Second, in the broadest reasonable interpretation of the term "pluripotent"

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includes pluripotent embryonic stem cells (ESCs) that have special properties that pluripotent germ stem cells do not have.

The specification teaches testis cells were collected from newborn (0-8 days old) ddY mice, DBA/2 mice or transgenic mouse line C57BL6/Tg14 (act-EGFP-Osby01) that was bred into DBA/2 background (designated Green) [0206]. Because these Green mice have the expressed the EGFP gene in substantially all cell types, it is possible to track the cells derived from the mice can be tracked with the fluorescence of EGFP as the indicator [0206]. The specification also teaches for some experiments, testis cells were collected from a newborn p53 deficient mouse in ICR background [0207]. The specification contemplates the pluripotent stem cells obtained by a living organism; all experimental techniques and methods applicable to ES cells or EG cells can be applied to the pluripotent stem cells; using the pluripotent stem cells, it is possible to produce diverse functional cells, tissues, animals (excluding humans) and the like [0186]. However, the specification fails to provide guidance for producing pluripotent stem cells as known in the art by forming the three somatic lineages that is by forming endoderm cells, ectoderm cells and mesoderm cells which pluripotent germ stem cells are derived from spermatogonial stem cells. The art teaches that it is unpredictable to produce pluripotent germ stem cells derived from all species of a postnatal mammal including a human. **Brinster** (Science, 316: 404-405, 2007 (IDS)) notes GDNF appears to be a primary regulator of the self-renewal versus differentiation fate decision for mouse and rat SSCs, and it is probably a conserved self-renewal signal for all mammalian SSCs and similar to embryonic stem cells (ESCs), SSCs grow in vitro on feeder cells in islands or clumps, and they stain positive for POU domain transcription factor 1 (Oct 3/4) and alkaline phosphatase (p 404, 3<sup>rd</sup> column). However, Brinster also notes that even SSCs might be pluripotent, however, SSCs are distinct from ESCs because ESCs readily generate teratocarcinomas when transplanted in vivo, while SSCs do not

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form tumors under similar conditions and whether the normal adult SSC can be induced to become pluripotent remains controversial. **Turnpenny et al**, (Stem Cells, 24: 212-220, 2006 (IDS)) note the influence of the basic media and feeder layers, all groups reporting hEGC derivation and culture have included other additives and their use originates from mouse pluripotent stem cell derivation and culture however, definitive requirements for any in equivalent hEGC cultures have yet to be established conclusively (p 215, 1<sup>st</sup> column, under media additives and critical factors). Moreover, Turnpenny et al note there are differences in pluripotent stem cells between mice and humans and despite activation of the LIFR/gp130-STAT3B pathway, recombinant human LIF does not maintain self-renewal of hESCs, which require feeder cells or their conditioned media with an extracellular matrix (p 215, 2<sup>nd</sup> column, last paragraph). Turnpenny et al note several groups have noted difficulty in maintaining hEGCs undifferentiated long-term and this problem of undifferentiated status contrasts with other pluripotent stem cell types: hESCs and human embryonal carcinoma cells (hECCs) and mESCs and mEGCs, all of which have been more extensively characterized (p 217, 1<sup>st</sup> column, 1<sup>st</sup> paragraph). Human EGC cultures have proliferated extensively; however, the proportion of cells expressing pluripotent markers (e.g., OCT4 and stage specific embryonic antigen [SSEA] family members declines over time, variably from 2 to 3 months onwards, and is exacerbated by freeze-thaw routines (p 217, 1<sup>st</sup> column, 1<sup>st</sup> paragraph). **Behrouz Aflatoonian et al** (Current Opinion in Biotechnology, 16: 530-535, 2005 (IDS)) note although the pluripotent and proliferative capacity of hEG cells is thought to be equivalent to that of human embryonic stem (hES) cells, there exist difficulties of isolating and maintaining hEG cell lines in vitro (abstract). Aflatoonian notes although the initial generation of hEG cells is relatively simple, the maintenance of well-defined cell lines through extended passage in culture has proved to be quite difficult to date (p 530 2<sup>nd</sup> column, 1<sup>st</sup> paragraph). Aflatoonian teaches for instance, just

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as differences are apparent in the production and maintenance of ES cells from the mouse and human there are also discrepancies in EG cell culture conditions for these species (p 532, 2<sup>nd</sup> column). Behrouz Aflatoonian suggests that it is unpredictable to obtain pluripotent stem cells from cultured human spermatogonial stem cells. For example, hES cells much prefer passage in culture as aggregated plaques if they are to remain undifferentiated (p 532, 2<sup>nd</sup> column). The complete desegregation of the human genital ridge is more difficult to achieve without compromising PGC survival and EG derivation (p 532, 2<sup>nd</sup> column). This property of human EG cells also extends to their proliferation where, instead of expanding as a relatively flat colony, hEG cells remain as a compacted mound of cells that is multi-layered and resistant to desegregation procedures and the capacity of human EG cells to be dissociated into small aggregates for passage, and remain viable and undifferentiated, is therefore very low, hence, the maintenance of hEG cell lines indefinitely and even beyond 15-20 passages is of practical importance if these cell lines are to be used for cell therapy (p 532, 2<sup>nd</sup> column bridge to p 533, 1<sup>st</sup> column).

In light of the above, the state of the art is suggesting that producing pluripotent stem cells from testis from all mammalian species of cultured testis cells containing spermatogonial stem cells might be feasible in the future. The instant specification does not provide any relevant teachings, specific guidance, or working examples for overcoming the limitations of maintaining pluripotency of human embryonic stem cells from postnatal testis by forming all three somatic cells lineages in any mammalian species or human raised by the state of the art. Applicants have not provided guidance to overcome the issue of maintaining viable and undifferentiated, pluripotent stem cells is very low, due to incomplete desegregation as raised in the art. Therefore, the skilled artisan would conclude that the state of art of producing pluripotent stem cells from postnatal testis is undeveloped and unpredictable at best. Given the lack of

guidance provided by the instant specification, it would have required undue experimentation to practice the invention as claimed for producing pluripotent stem cells from postnatal testis from all mammalian species without a reasonable expectation of success.

Therefore, in view of the quantity of experimentation necessary to determine the parameters listed above for producing pluripotent stem cells derived from postnatal testis of all mammalian species including human species, forming the three somatic lineages, the lack of direction or guidance provided by the specification for producing pluripotent stem cells derived from postnatal testis of all mammalian species including human species, forming the three somatic lineages, the absence of working examples for producing pluripotent stem cells derived from postnatal testis of all mammalian species including human species, forming the three somatic lineages, the unpredictable state of the art with respect to producing pluripotent stem cells derived from postnatal testis of all mammalian species including human species, forming the three somatic lineages, the undeveloped state of the art pertaining to producing pluripotent stem cells derived from postnatal testis of all mammalian species including human species, forming the three somatic lineages, and the breadth of the claims directed to all mammalian species, it would have required undue experimentation for one skilled in the art to make and/or use the claimed invention.

A. Applicants argue the claimed method comprises the derivation of pluripotent stem cells from postnatal testis cells comprising spermatogonial stem cells under specific conditions. Applicants argue embryonic stem cells (ES cells) are entirely different from the spermatogonial stem cells recited in the pending claims. ES cells are well-known pluripotent stem cells produced by in vitro cultivation of an inner cell mass. The guidance provided in the specification regarding the age of the postnatal mammal makes it clear that the postnatal mammal does not contain ES cells (see, e.g., page 15, line 34, through page 16, line 22, of the



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specification).

These arguments are not persuasive because they are based on the broadest reasonable interpretation of the term “pluripotent” as instantly claimed in the independent claim 1 pluripotent cells embraces embryonic stem cells which as discussed above GDNF regulates growth of ESCs in culture ESCs which can readily generate teratocarcinomas when transplanted in vivo (see above Brinster et al). In contrast, to ESCs, the SSCs have characteristics that are not present in the ESCs such as SSCs do not form tumors under similar conditions as ESCs as taught by Brinster et al and as discussed above.

B. Applicants argue the specification describes the problems associated with the production of pluripotent stem cells from adult testis and specific methods to solve the problems. In particular, the specification identifies the low frequencies of stem cells in adult testis as a significant problem to be solved in the production of pluripotent stem cells from adult testis cells. The specification provides specific guidance as to methods of solving this problem by using a younger animal because younger animals have higher frequencies of stem cells (e.g., spermatogonial stem cells) contained in the testis (see, e.g., page 14, line 23, through page 15, line 5, and page 16, line 3-6), and using a cell sorter or antibody magnetic microbeads with an antibody-recognizing cell surface antigen specifically expressed in the spermatogonial stem cells (see, e.g., page 14, line 29, through page 15, line 2).

These arguments are not persuasive because producing pluripotent stem cells from testis cells derived from a postnatal mammal including younger animals embrace pluripotent stem cells derived from embryonic stem cells. As discussed above the spermatogonial stem cells acquire markers of the spermatogonial lineage while the embryonic stem cells derived from the ICM lack said lineage markers.

C. Applicants argue the Guan 2006 reference discloses that testis cells containing Stra8 positive spermatogonial stem cells from adult mice cultured in a medium containing GDNF result in the formation of testicular teratomas (tumors derived from pluripotent cells) at low frequencies (see, e.g., page 1199, column 1, lines 23-41). The Guan 2009 reference discloses the isolation of testis cells from 2 to 5 week old mice (see page 143, "Isolation of testicular cells"), the enrichment of spermatogonial stem cells from testis cells in various ways (see page 143, "Methods for enriching for SSCs"), culturing in a medium containing GDNF (page 143, "Culturing SSCs"), and the appearance of ES-like colonies of maGSCs (pluripotent stem cells) at 113 days (or at least 4-8 weeks) after initiation of the culture (see page 149, "Conversion of S SCs into maGSCs" and Figure 2C).

These arguments are not persuasive because Guan teaches SSCs themselves may be multipotent (p 1202, 1<sup>st</sup> column, last paragraph). Guan suggests when SSCs are expanded in the absence of Sertoli cells, *in vitro* culture and in blastocysts, some SSCs might be released from this inhibition and converted into pluripotent stem cells (p 1202, 1<sup>st</sup> column, last paragraph). Therefore, Guan does not teach the derivation of pluripotent stem cells from testis cells containing ESCs as embraced in the instant claimed invention. Regarding Guan (2009) reference like Guan 2006 teaches an a modified method of the isolation of maGSCs and for the same reasons as discussed above does not teach the derivation of pluripotent stem cells from testis cells containing ESCs as instantly embraced in the claimed invention.

D. Applicants argue the Conrad reference discloses the enrichment of spermatogonial cells from adult human testis using MACS with an antibody against  $\alpha 6$ -integrin, culturing in a medium containing GDNF and LIF or GDNF alone for as long as 42 days to develop clusters of GSCs (pluripotent stem cells), and the formation of teratomas after injection

of the resultant human adult GSEs into an immunodeficient mouse (see, e.g., Figures 2 and 5, and page 5, column 1, lines 13-30). Accordingly, the post-filing Guan and Conrad references further evidence the success of the inventive method using adult mice and human testis cells.

These arguments are not persuasive because Conrad does not teach the derivation of pluripotent stem cells from testis cells containing ESCs as embraced in the instant claimed invention.

E. Applicants argue regarding Turnpenney et al., Stem Cells, 24:212-220 (2006), the Office alleges that the culture of embryonic germ (EG) cells from different species is controversial and unpredictable. The Office believes that the proliferation of mouse spermatogonial stem cells is dependent on LIF and not GDNF.

These arguments are not persuasive because Turnpenny teaches additional issues regarding the culturing of embryonic germ cells from different species as a source of spermatogonial germ cells as instantly required for the culture of said cells to obtain pluripotent cells. Turnpenny and brings the issue of the differences in pluripotent stem cells between mice and humans, wherein for example, despite activation of the LIFR/gp130-STAT3B pathway, LIF does not maintain self-renewal of hESCs, which require feeder cells or their conditioned media with an extracellular matrix. Therefore, the culture of embryonic germ cells from different species is controversial and unpredictable at best.

F. Applicants argue regarding Aflatoonian et al., Curr. Opin. Biotech., 16: 530- 535 (2005), describes the difficulty of maintaining well-defined human EG cell lines through extended passage in culture, even though the initial generation of human EG cells is relatively simple. Applicants note that the pending claims are directed to a method of producing (i.e.,

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generating) pluripotent stem cells from testis cells, and not to a method of maintaining human EG cells in culture over multiple passages. However, even if the maintenance of human EG cells is difficult, Applicants note that human EG cells have been established as described in Turnpenney et al., Stem Cells, 21:598-609 (2003).

These arguments are not persuasive because the Turnpenney et al., Stem Cells, 21:598-609 (2003) reference teaches derivation of human embryonic germ cells as an alternative source of pluripotent stem cells from embryos unlike the instantly claimed postnatal derived cells (adult testis cells) (see p 599, under materials and methods).

***Claim Rejections - 35 USC § 103/Necessitated by Amendment***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims **1-2, 4-6, 15, 35-36** are rejected under 35 U.S.C. 103 (a) as being unpatentable over **Hogan** (US 5,690,926 (IDS)) in view of **Creemers et al**, (Reproduction, 124: 791-799, 2002 (IDS)); **Van Pelt et al** (Endocrinology, 143(5):1845-50, 2002); **Tadokoro et al**, (Mechanisms of Development, 113: 29-39, 2002 (IDS)); **Meng et al** (Science, 287: 1489-1493, 2000 (IDS)).

**Hogan** teaches a method for the isolation of embryonic stem cell lines from postnatal mammalian testis in the presence of feeder cells, leukemia inhibitory factor (LIF) soluble stem cell factor, and basic fibroblast factor (column 12, lines 61-67 bridge column 13, lines 1-13)

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**(claim 1).** Hogan discloses a method of making a mammalian pluripotent embryonic stem cell comprising culturing postnatal mammalian testis in a composition comprising a growth enhancing amount of basic fibroblast growth factor, leukemia inhibitory factor, membrane associated steel factor, and soluble steel factor, thereby making a pluripotent embryonic stem cell from a germ cell (column 6, lines 62-67 bridge to column 7, lines 1-3). Hogan discloses "Germ cells" as used herein means the cells which exist in neonatal or postnatal testis and are the progenitors of gametes. In the testis, these germ cells represent a small population of stem cells capable of both self-renewal and differentiation into mature spermatogonia. Thus, "germ cells" are the postnatal equivalent to the prenatal primordial germ cells and can include primitive or immature spermatogonia such as type A spermatogonia or any undifferentiated early stage cell that can form a pluripotent embryonic stem cell (column 7, lines 3-12) (**claim 6** of the instant invention). Hogan also teaches these methods can be practiced utilizing mammal cells including mice, rats, rabbits, guinea pigs, goats, cows, pigs, humans (column 7, lines 13-14) (**claims 35-36**). Hogan teaches to determine whether PGCs and their descendants continue to proliferate in culture, primary colonies of PGCs were trypsinized after 6 days in culture and replated on a fresh Sl.sup.4 -m220 feeder layer with added growth factors. By day 6 in secondary culture, large colonies of densely packed alkaline positive (AP) positive cells resembling embryonic stem (ES) cells are present (FIG. 2D, E; FIG. 4, A), and these colonies are also positive for the expression of the antigen SSEA-1, a characteristic of PGCs (column 9, lines 61-67 bridge to column 10) (**claims 2-6, 15** of the instant invention). Hogan differs from the present invention for not teaching glial cell derived neurotrophic factor (GDNF) for producing pluripotent stem cells from postnatal testis.

However, at the time of the instant invention **Creemers et al** disclose culturing testes cells from testes removed from Nc/CpbU adult or prepubertal mice and isolating type A

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spermatogonia (p 792, 2<sup>nd</sup> column, 2<sup>nd</sup> paragraph). Creemers teaches the type A spermatogonia cells under cultured conditions by adding GDNF to the basic culture media containing bFGF and LIF (p 792 2nd column bridges to p 793, 1<sup>st</sup> column). Creemers teaches a suspension of type A spermatogonia of which about 10% consists of spermatogonial stem cells, normally comprising only 0.03% of the testicular germ cell population (p 792, 1<sup>st</sup> column). Creemers teaches comparison of cultures of adult cells with cultures of prepubertal germ cells, commonly used in studies of spermatogenesis, showed that prepubertal germ cells are twice as viable (abstract). Creemers teaches the viability and proliferation of cultured cells peaks at day 1 and decreases over a period of 7 days (see figures 2, 5 and 6). Creemers also teaches at day 7 an extensive layer of flattened epithelial-like cells had formed where these flattened cells were shown to incorporate BrdU and on top of this layer a few small, round, Hsp90a-positive cells are present of which none was positive for BrdU (see page 795 column 1-2 and figure 7). Creemers suggests the actual percentage of 58% fro prepubertal mouse germ cells in the present study seems to represent a moderate improvement and additional refinement by adjusting the growth factors concentrations might further enhance viability (p 798, 1<sup>st</sup> column 2nd paragraph). Creemers suggests optimization of the culture of germ cells in defined medium could be achieved by several approaches including the addition to the culture medium the appropriate concentrations of growth factors that might greatly improve viability or proliferation of spermatogonia and the use of well-defined culture conditions for culturing early germ cells is needed for many purposes and merits further studies (p 798, 1<sup>st</sup> column last paragraph). Creemers differs from the present invention for not teachings the growth of spermatogonial stem cells for at least 3 to 6 weeks.

However, at the time of the instant invention **Van Pelt et al** (Endocrinology, 143(5):1845-50, 2002) teaches long term culture of rat spermatogonial stem cells which are positive for both

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spermatogonial stem cell markers Hsp90alpha and oct-4 cultured for 3 yrs and passaged more than 50 times without any morphological changes (p 1846, 2<sup>nd</sup> column under establishment of cell lines). In addition, Van Pelt teaches the cells still are able to proliferate even after the cultures become confluent and overgrow each other, showing low contact inhibition (p 1846, 2<sup>nd</sup> column under establishment of cell lines). Van Pelt suggests these first spermatogonial stem cell can be used to study spermatogonial gene expression in comparison with more advanced germ cells (abstract). **Tadokoro et al**, (Mechanisms of Development, 113: 29-39, 2002) teach that high levels of GDNF are necessary for the proliferation of all undifferentiated spermatogonial stem cells (abstract). In addition, Tadokoro teaches Oct-3/4-positive and negative cells are likely to be reversible and the active proliferation seen in both subpopulations suggests that all undifferentiated spermatogonia enter the cell cycle to recover from the impaired state, because cell proliferation was controlled by GDNF stimulation, (figure 3, p 35, 1<sup>st</sup> column, 1<sup>st</sup> paragraph). **Meng et al** (Science, 287: 1489-1493, 2000) supplements the teachings of Tadokoro by teaching GDNF at high concentrations favors self-renewal of undifferentiated spermatogonial stem cells (abstract, p 1492, 2<sup>nd</sup> column, 1<sup>st</sup> paragraph).

The combination of prior art cited above in all rejections under 35 U.S.C. 103 satisfies the factual inquiries as set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966). Once this has been accomplished the holdings in KSR can be applied (*KSR International Co. v. Teleflex Inc.* (KSR), 550 U.S. \_\_\_, 82 USPQ2d 1385 (2007): "Exemplary rationales that may support a conclusion of obviousness include: (A) Combining prior art elements according to known methods to yield predictable results; (B) Simple substitution of one known element for another to obtain predictable results; (C) Use of known technique to improve similar devices (methods, or products) in the same way; (D) Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results; (E)

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“Obvious to try” – choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success; (F) Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art; (G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.”

Accordingly, it would have been obvious to the ordinarily skilled artisan to modify the teachings of Hogan to utilizing GDNF for culturing the spermatogonial stem cell as taught by Creemers and further to use high concentrations of GDNF as taught by Tadokoro and Meng, with a reasonable expectation of success. One of ordinary skill in art would have been motivated to use high concentration of GDNF in the culture system of spermatogonial stem cells of Creemers in order to recover the Hsp90alpha non-proliferating BrdU negative spermatogonial stem cells of Creemers at day 7 since Tadokoro teach that high levels of GDNF is necessary for the proliferation of all undifferentiated spermatogonial stem cells such as Oct-3/4-positive and since Meng teaches high concentrations of GDNF favors self renewal over differentiation of spermatogonial stem cells. This is further underscored by the teachings of Van Pelt who teach that long term culture of spermatogonial stem cells need both spermatogonial stem cell markers Hsp90alpha and oct-4 cultured and since Tadokoro teach high GDNF concentrations induce Oct 4 expression. Moreover, one of skill in the art would have particularly motivated to use high GDNF levels in the culture system of Hogan/Creemers for recovering the Hsp90alpha positive non-proliferating spermatogonial stem cells in order to culturing for longer than 7 days since Tadokoro suggests that all undifferentiated spermatogonia enter the cell cycle to recover from the impaired state, because cell proliferation was controlled by GDNF stimulation, (figure 3, p



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35, 1<sup>st</sup> column, 1<sup>st</sup> paragraph) and since Creemers suggests optimization of the culture of germ cells in defined medium could be achieved by several approaches including the addition to the culture medium the appropriate concentrations of growth factors that might greatly improve viability or proliferation of spermatogonia stem cells.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims **1, 3** are rejected under 35 U.S.C. 103 (a) as being unpatentable over Hogan (US 5,690,926 (IDS)) in view of Creemers et al, (Reproduction, 124: 791-799, 2002 (IDS)); Van Pelt et al (Endocrinology, 143(5):1845-50, 2002); Tadokoro et al, (Mechanisms of Development, 113: 29-39, 2002); Meng et al (Science, 287: 1489-1493, 2000 (IDS)) and further in view of **Haneji et al** (J Endocrinology, 128(3): 383-8, 1991 (IDS)) taken with **Wahab-Wahlgren** (Mol and Cell Endocrin, 201: 39-46, 2003 (IDS)).

The teachings of Hogan/Creemers/Van Pelt/ Tadokoro/Meng apply here as stated above.

Hogan/Creemers/Van Pelt/ Tadokoro/Meng do not teach EGF in culture media for producing pluripotent stem cells from postnatal testes.

However, at the time of the instant invention Haneji teaches that different concentrations of EGF inhibits the differentiation of type A spermatogonia (p 385, 1st column, figure 2). Wahab-Wahlgren teaches that EGF plays an important role in spermatogonia proliferation in vitro (abstract, and p 44, 1<sup>st</sup> column, 1<sup>st</sup> paragraph, discussion).

The combination of prior art cited above in all rejections under 35 U.S.C. 103 satisfies the factual inquiries as set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459

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(1966). Once this has been accomplished the holdings in KSR can be applied (*KSR International Co. v. Teleflex Inc.* (KSR), 550 U.S. \_\_\_, 82 USPQ2d 1385 (2007): “Exemplary rationales that may support a conclusion of obviousness include: (A) Combining prior art elements according to known methods to yield predictable results; (B) Simple substitution of one known element for another to obtain predictable results; (C) Use of known technique to improve similar devices (methods, or products) in the same way; (D) Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results; (E) “Obvious to try” – choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success; (F) Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art; (G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.”

Accordingly, it would have been obvious for one of ordinary of skill in the art to modify the technology of culturing pluripotent spermatogonial stem cells as taught by the combined references of Hogan/Creemers/Van Pelt/ Tadokoro/Meng to include EGF as taught by Haneji taken with Wahab-Wahlgren, with a reasonable expectation of success. One d ordinary of skill in the art would have been motivated to make this modification because Haneji teaches EGF inhibits differentiation of type A spermatogonia. One d ordinary of skill in the art would have been particularly motivated to make this modification in order to maintain the pluripotency of the spermatogonia stem cells.

Thus, the claimed invention as a whole, is clearly prima facie obvious in the absence of evidence to the contrary.

Claims **1, 7** are rejected under 35 U.S.C. 103 (a) as being unpatentable over Hogan (US 5,690,926 (IDS)) in view of Creemers et al, (Reproduction, 124: 791-799, 2002); Van Pelt et al (Endocrinology, 143(5):1845-50, 2002); Tadokoro et al, (Mechanisms of Development, 113: 29-39, 2002 (IDS)); Meng et al (Science, 287: 1489-1493, 2000 (IDS)); Haneji et al (J Endocrinology, 128(3): 383-8, 1991 (IDS)) taken with Wahab-Wahlgren (Mol and Cell Endocrin, 201: 39-46, 2003).and further in view **Beumer et al** (Cell Death and Differentiation , 5: 669-677, 1998 (IDS)).

The teachings of Hogan/Creemers/Van Pelt/ Tadokoro/Meng/ Haneji/Wahab-Wahlgren is applied here as indicated above.

Hogan/Creemers/Van Pelt/ Tadokoro/Meng/ Haneji/Wahab-Wahlgren, do not teach the testis cells are p53-deficient.

However, at the time the invention was made, Beumer et al teach spermatogonia cell production by the undifferentiated spermatogonia is much more efficient in p53 knock out mice than in wild-type mice, indicating enhanced proliferative activity or less apoptosis of these cells (p 675, 1<sup>st</sup> column, 1<sup>st</sup> paragraph). Beumner is an exemplified prior art that teaches that it is routine or well-established in the art to employ p53-deficient testis cells as a source of obtaining SSCs since p53 knock out mice, constitute an increased numbers of spermatogonia (p 670, 1<sup>st</sup>column, 1<sup>st</sup> paragraph).

The combination of prior art cited above in all rejections under 35 U.S.C. 103 satisfies the factual inquiries as set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966). Once this has been accomplished the holdings in KSR can be applied (*KSR International Co. v. Teleflex Inc. (KSR)*, 550 U.S. \_\_\_, 82 USPQ2d 1385 (2007): "Exemplary rationales that may support a conclusion of obviousness include: (A) Combining prior art

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elements according to known methods to yield predictable results; (B) Simple substitution of one known element for another to obtain predictable results; (C) Use of known technique to improve similar devices (methods, or products) in the same way; (D) Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results; (E) "Obvious to try" – choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success; (F) Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art; (G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention."

Accordingly, it would also have been obvious for one of ordinary skill in the art of isolating pluripotent stem cells from mouse testis to further employ p53 knock testis from p53 knock out mice of choice available in the art in order to obtain pluripotent type A spermatogonial stem cells SSCs from postnatal cultured testis cells of the combined cited references. One of ordinary skill in the art would have been motivated to employ p53 deficient testis cells in the system of Hogan/Creemers/Van Pelt/ Tadokoro/Meng/ Haneji/Wahab-Wahlgren, in order to increase the number of undifferentiated SSCs as taught by Beumer. One of ordinary skill in the art would have reasonably expected that inclusion of p53 deficient testis cells are routinely employed in the art and can help to further isolate pluripotent stem cells from mouse testis particularly in view of the totality of the prior art at the time the invention was made.

Thus, the claimed invention as a whole, is clearly prima facie obvious in the absence of evidence to the contrary.

Claims **1, 16** are rejected under 35 U.S.C. 103 (a) as being unpatentable over **Hogan** (US 5,690,926 (IDS)) in view of **Creemers et al**, (Reproduction, 124: 791-799, 2002); **Van Pelt et al** (Endocrinology, 143(5):1845-50, 2002); **Tadokoro et al**, (Mechanisms of Development, 113: 29-39, 2002); **Meng et al** (Science, 287: 1489-1493, 2000) and further in view of **Kanatsu-Shinohara et al**, [Biology of Reproduction 70, 70–75 ,2004, (IDS)]; **Shinohara et al**, [PNAS, 96: 5504-5509, 1999, (IDS)]; **Van Der Wee et al** (Journal of Andrology, 22(4): 696-704, 2001 (IDS)).

The teachings of Hogan/Creemers/Van Pelt/Tadokoro/Meng is applied here as indicated above.

Hogan/Creemers/Van Pelt/Tadokoro/Meng do not teach the pluripotent stem cells are positive for CD9,  $\beta 1$ - and  $\alpha 6$ -integrin, Ep-CAM and c-kit surface markers.

However, at the time of the instant invention Kanatsu-Shinohara et al, teach spermatogonial stem cells can be enriched by selection with an antibody against cell-surface molecules. Kanatsu-Shinohara teaches spermatogonial stem cells express the surface CD9 molecule, which is commonly expressed on stem cells of other tissues (abstract). Selection of both mouse and rat testis cells with anti-CD9 antibody resulted in 5- to 7-fold enrichment of spermatogonial stem cells from intact testis cells, indicating that CD9 is commonly expressed on spermatogonial stem cells of both species (abstract). Therefore, CD9 may be involved in the common machinery in stem cells of many self-renewing tissues, and the identification of a common surface antigen on spermatogonial stem cells of different species has important implications for the development of a technique to enrich stem cells from other mammalian species (abstract). As such Kanatsu-Shinohara et al, provide sufficient motivation to enrich pluripotent stem cells by the use of the CD9 marker as taught by Kanatsu-Shinohara et al. Shinohara et al, supplements the teachings of Kanatsu-Shinohara et al, by teaching SSCs also

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express the surface markers  $\beta 1$ - and  $\alpha 6$  integrin (title). Shinohara suggests the degree of enrichment of stem cells attainable by this method will allow further fractionation and analysis of the enriched cell population to identify a set of additional antigens characteristic of and unique for spermatogonial stem cells (p 5509, 1<sup>st</sup> column, 1<sup>st</sup> paragraph). A systematic evaluation of surface molecules on the stem cell will facilitate identification and purification of these cells and greatly contribute to our understanding of their biology and survival requirements. This approach mirrors developments made with hematopoietic stem cells during the last decade. As stem cell markers are identified, enrichment for stem cell populations can be increased, facilitating their eventual purification. **Van Der Wee** teaches the isolation of type A spermatogonia using magnetic beads and antibodies that recognize the c-kit receptor or the Ep-CAM (abstract).

The combination of prior art cited above in all rejections under 35 U.S.C. 103 satisfies the factual inquiries as set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966). Once this has been accomplished the holdings in KSR can be applied (*KSR International Co. v. Teleflex Inc. (KSR)*, 550 U.S. \_\_\_, 82 USPQ2d 1385 (2007): “Exemplary rationales that may support a conclusion of obviousness include: (A) Combining prior art elements according to known methods to yield predictable results; (B) Simple substitution of one known element for another to obtain predictable results; (C) Use of known technique to improve similar devices (methods, or products) in the same way; (D) Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results; (E) “Obvious to try” – choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success; (F) Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art; (G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to

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modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.”

Accordingly, in view of the teachings of Kanatsu-Shinohara/Shinohara/ **Van Der Wee**, it would have been obvious for one of ordinary of skill in the art to use the SSC surface markers of CD9,  $\beta$ 1- and  $\alpha$ 6 integrin, c-kit and Ep-CAM for the production of enriched pluripotent stem cells from testis cells by modifying the system of the combined references in the art. One of ordinary of skill in the art would have been sufficiently motivated for such a modification in order to produce enriched pluripotent stem cells from testicular SSCs via cell surface markers using fluorescence sorting from all different species particularly in view of the totality of the prior art at the time the invention was made. Even though, the combined references do not teach the Forsman antigen and EE2 marker the cultured pluripotent cells of the combined cited references would inherently carry said surface markers.

Thus, the claimed invention as a whole, is clearly prima facie obvious in the absence of evidence to the contrary.

Applicants argue that the combined teachings of Hogan and Creemers do not teach culture of spermatogonial stem cells in culture for more than 7 days.

These arguments are not persuasive because the combined teachings of **Hogan** in view of **Creemers et al**; **Van Pelt et al**; **Tadokoro et al**; **Meng et al** (Science, 287: 1489-1493, 2000) teach the culture of the spermatogonial stem cells for long term up to 3 yrs.

### ***Conclusion***

**No claim is allowed.**

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Magdalene K. Sgagias whose telephone number is (571) 272-3305. The examiner can normally be reached on Monday through Friday from 9:00 am to 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras, Jr., can be reached on (571) 272-4517. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

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